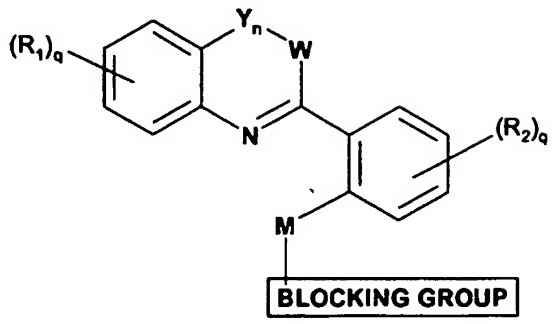
Claims

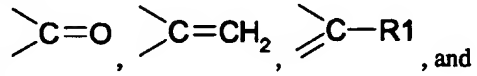
1. An enzyme substrate of the formula (I):



(I)

and biologically acceptable salts, and pro-reporter molecules thereof;

wherein

Y is  , and

n is 1 or 0;

W is =CH-, -S-, -O-, or -N(R_3)-;

M is oxygen, nitrogen or sulfur;

R_1 and R_2 are, each independently, hydrogen, halogen, nitro, azido, mercapto, sulfeno, sulfino, sulfo, cyano, amino, R_4-, R_4O-, $R_4C(=Z)$-, R_4X-C(=Z)-, R_4-C(=Z)-X-, R_4X-C(=Z)-Q-, R_4S-, R_4-S(=O)-, R_4-S(=O)-O-, R_4-S(=O)$_2$-O-, R_4O-S-, R_4O-S(=O)-, R_4O-S(=O)$_2$-, R_4R_5N-S(=O)-, R_4R_5N-S(=O)$_2$-, R_4R_5N-, [R_4-C(=Z)][R_5]N-, [R_4-C(=Z)][R_5-C(=X)]N-, R_4R_5N-C(=Z)-, R_4R_5N-C(=Z)-X-, [R_4R_5N-C(=Z)][R_6]N-, [R_4R_5N-C(=Z)][R_6-C(=X)]N-, [R_4-S(=O)][R_5]N-, [R_4-S(=O)$_2$][R_5]N-, (R_4X)(R_5Q)P(=Z)-, (R_4R_5N)(R_6X)P(=Z)-, (R_4R_5N)(R_6R_7N)P(=Z)-, (R_4X)(R_5Q)P(=Z)-O-, (R_4R_5N)(R_6X)P(=Z)-O-, (R_4R_5N)(R_6R_7N)P(=Z)-O-, (R_4X)(R_5Q)P(=Z)-S-, (R_4R_5N)(R_6X)P(=Z)-S-, (R_4R_5N)(R_6R_7N)P(=Z)-S-, [(R_4X)(R_5Q)P(=Z)][R_6]N-, [(R_4R_5N)(R_6X)P(=Z)][R_7]N-, [(R_4R_5N)(R_6R_7N)P(=Z)][R_8]N-, (R_4)(R_5X)P(=Z)-O-, (R_4)(R_5R_6N)P(=Z)-O-, (R_4)(R_5X)P(=Z)-S-, (R_4)(R_5R_6N)P(=Z)-S-, [(R_4)(R_5X)P(=Z)][R_6]N-, [(R_4)(R_5R_6N)P(=Z)][R_7]N-;

wherein **X, Z and Q** are each independently oxygen or sulfur;

R_3 is R_4, R_4-C(=Z)-, R_4X-C(=Z)-, R_4R_5N-C(=Z)-, R_4O-S(=O)-, R_4O-S(=O)$_2$-, R_4R_5N-S(=O)-, R_4R_5N-S(=O)$_2$-, $(R_4X)(R_5Q)P(=Z)$-, $(R_4R_5N)(R_6X)P(=Z)$-, $(R_4R_5N)(R_6R_7N)P(=Z)$-;

wherein R_4, R_5, R_6, R_7 and R_8 are, each independently, hydrogen, C_{1-8}alkyl, C_{2-8}alkenyl, C_{2-8}alkynyl, C_{3-7}cycloalkyl, aryl, Het^1, Het^2;

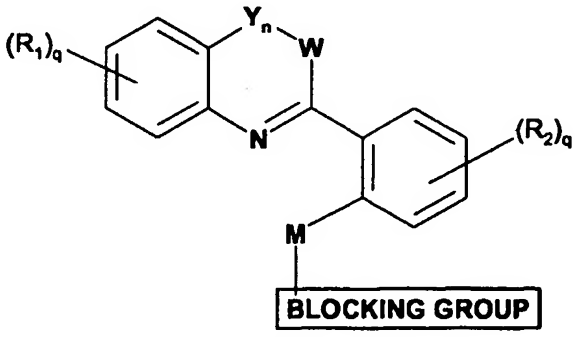
each q is independently 0, 1, 2, 3, or 4;

wherein any C_{1-8}alkyl, C_{2-8}alkenyl, C_{2-8}alkynyl or amino group, may be further mono, di-, or tri- substituted (if the valency allows it) with C_{1-4}alkoxy, C_{1-4}alkylcarbonyl, C_{1-4}alkoxycarbonyl, C_{1-4}alkylthio, C_{1-4}alkylsulfenyl, C_{1-4}alkylsulfinyl, C_{1-4}alkylsulfonyl, C_{1-4}alkylamino, halogen, nitro, azido, mercapto, sulfeno, sulfino, sulfo, cyano, amino, aryl, Het^1 or Het^2 substituents;

wherein the **BLOCKING GROUP** is a mono- or polysaccharide derivate, phosphate derivate, or sulfate derivate;

with the proviso that at least one R_1, R_2 and R_3 is a moiety with at least 4 carbons.

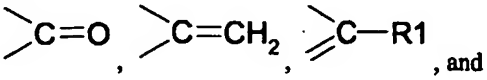
2. An enzyme substrate of the formula (I):



(I)

and biologically acceptable salts and pro-reporter molecules thereof;

wherein

Y is  , and

n is 1 or 0;

W is =CH-, -S-, -O-, or -N(R_3)-;

M is –O-, -N(R_3)-, or –S-;

each R_1 and each R_2 present in formula (I) are, independently, hydrogen, halogen, nitro, azido, mercapto, sulfeno, sulfino, sulfo, cyano, amino, R_4-, R_4O-, R_4-C(=Z)-, R_4X-C(=Z)-, R_4-C(=Z)-X-, R_4X-C(=Z)-Q-, R_4S-, R_4-S(=O)-, R_4-S(=O)$_2$-, R_4-S(=O)-O-, R_4-S(=O)$_2$-O-, R_4O-S-, R_4O-S(=O)-, R_4O-S(=O)$_2$-, R_4R_5N-S(=O)-, R_4R_5N-S(=O)$_2$-, R_4R_5N-, [R_4-C(=Z)][R_5]N-, [R_4-C(=Z)][R_5-C(=X)]N-, [R_4X-C(=Z)][R_5]N-, [R_4X-C(=Z)][R_5-C(=Q)]N-, R_4R_5N-C(=Z)-, R_4R_5N-C(=Z)-X-, [R_4R_5N-C(=Z)][R_6]N-, [R_4R_5N-C(=Z)][R_6C(=X)]N-, [R_4-S(=O)][R_5]N-, [R_4-S(=O)$_2$][R_5]N-, (R_4X)(R_5Q)P(=Z)-, (R_4R_5N)(R_6X)P(=Z)-, (R_4R_5N)(R_6R_7N)P(=Z)-, (R_4X)(R_5Q)P(=Z)-O-, (R_4R_5N)(R_6X)P(=Z)-O-, (R_4R_5N)(R_6R_7N)P(=Z)-O-, (R_4X)(R_5Q)P(=Z)-S-, (R_4R_5N)(R_6X)P(=Z)-S-, (R_4R_5N)(R_6R_7N)P(=Z)-S-, [(R_4X)(R_5Q)P(=Z)][R_6]N-, [(R_4R_5N)(R_6X)P(=Z)][R_7]N-, [(R_4R_5N)(R_6R_7N)P(=Z)][R_8]N-, (R_4)(R_5X)P(=Z)-O-, (R_4)(R_5R_6N)P(=Z)-O-, (R_4)(R_5X)P(=Z)-S-, (R_4)(R_5R_6N)P(=Z)-S-, [(R_4)(R_5X)P(=Z)][R_6]N-, [(R_4)(R_5R_6N)P(=Z)][R_7]N-;

wherein **X, Z and Q** are each, independently, O or S;

R_3 is R_4, R_4-C(=Z)-, R_4X-C(=Z)-, R_4R_5N-C(=Z)-, R_4O-S(=O)-, R_4O-S(=O)$_2$-, R_4R_5N-S(=O)-, R_4R_5N-S(=O)$_2$-, (R_4X)(R_5Q)P(=Z)-, (R_4R_5N)(R_6X)P(=Z)-, (R_4R_5N)(R_6R_7N)P(=Z)-;

wherein R_4, R_5, R_6, R_7 and R_8 are each, independently, hydrogen, C_{1-8}alkyl, C_{2-8}alkenyl, C_{2-8}alkynyl, C_{3-7}cycloalkyl, aryl, Het^1, Het^2;

each **q** present in formula (I) is, independently, 0, 1, 2, 3, or 4;

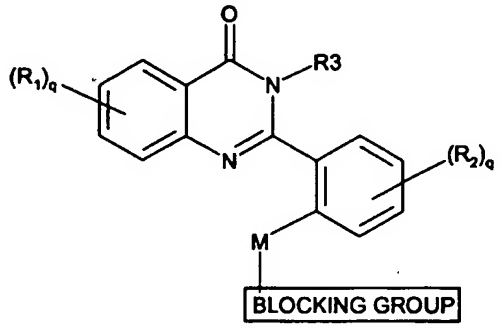
wherein any C_{1-8}alkyl, C_{2-8}alkenyl, C_{2-8}alkynyl or amino group, may be further mono, di-, or tri- substituted (if the valency allows it) with C_{1-4}alkoxy, C_{1-4}alkylcarbonyl, C_{1-4}alkoxycarbonyl, C_{1-4}alkylthio, C_{1-4}alkylsulfenyl, C_{1-4}alkylsulfinyl, C_{1-4}alkylsulfonyl, C_{1-4}alkylamino, halogen, nitro, azido, mercapto, sulfeno, sulfino, sulfo, cyano, amino, aryl, Het^1 or Het^2 substituents;

wherein the **BLOCKING GROUP** is a mono- or polysaccharide derivate, phosphate derivate, sulfate derivate, carboxylic acid derivate, or oligopeptide derivate;

with the proviso that at least one of R_1, R_2 and R_3 is C_{4-8}alkyl, C_{4-8}alkenyl, or C_{4-8}alkynyl.

3. A substrate according to any one of claims 1 to 2, wherein at least one of R_1, R_2 and R_3 is independently chosen from the group consisting of straight and branched butyl, pentyl, hexyl, heptyl, octyl.

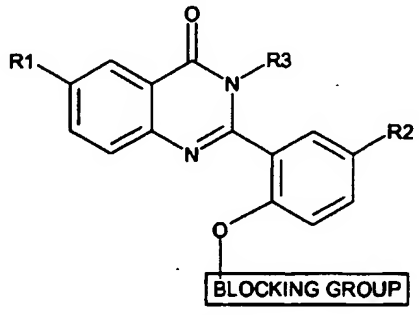
4. A substrate according to any one of claims 1 to 3, wherein W is -N(R3)-, Y is -C(=O)-, and n is 1 and having the formula (II)



(II)

wherein M, R_1, R_2, R_3, q and the **BLOCKING GROUP** are as defined as in any one of claims 1 to 3.

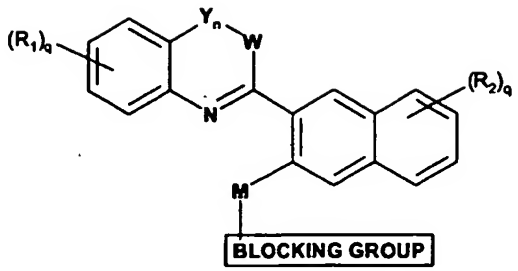
5. A substrate according to any one of claims 1 to 4, having the formula (III)



(III)

wherein R_1, R_2, R_3, and the **BLOCKING GROUP** are as defined as in any one of claims 1 to 3.

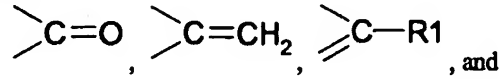
6. An enzyme substrate of the formula (V):



(V)

and biologically acceptable salts, and pro-reporter molecules thereof;

wherein

Y is  , and

n is 1 or 0;

W is =CH-, -S-, -O-, or $-N(R_3)-$;

M is –O-, $-N(R_3)-$, or –S-;

each R_1 and each R_2 present in formula (V) are, independently, hydrogen, halogen, nitro, azido, mercapto, sulfeno, sulfino, sulfo, cyano, amino, R_4-, R_4O-, R_4-C(=Z)-, R_4X-C(=Z)-, R_4-C(=Z)-X-, R_4X-C(=Z)-Q-, R_4S-, R_4-S(=O)-, R_4-$S(=O)_2$-, R_4-S(=O)-O-, R_4-$S(=O)_2$-O-, R_4O-S-, R_4O-S(=O)-, R_4O-$S(=O)_2$-, R_4R_5N-S(=O)-, R_4R_5N-$S(=O)_2$-, R_4R_5N-, [R_4-C(=Z)][R_5]N-, [R_4-C(=Z)][R_5-C(=X)]N-, [R_4X-C(=Z)][R_5]N-, [R_4X-C(=Z)][R_5-C(=Q)]N-, R_4R_5N-C(=Z)-, R_4R_5N-C(=Z)-X-, [R_4R_5N-C(=Z)][R_6]N-, [R_4R_5N-C(=Z)][R_6C(=X)]N-, [R_4-S(=O)][R_5]N-, [R_4-$S(=O)_2$][R_5]N-, (R_4X)(R_5Q)P(=Z)-, (R_4R_5N)(R_6X)P(=Z)-, (R_4R_5N)(R_6R_7N)P(=Z)-, (R_4X)(R_5Q)P(=Z)-O-, (R_4R_5N)(R_6X)P(=Z)-O-, (R_4R_5N)(R_6R_7N)P(=Z)-O-, (R_4X)(R_5Q)P(=Z)-S-, (R_4R_5N)(R_6X)P(=Z)-S-, (R_4R_5N)(R_6R_7N)P(=Z)-S-, [(R_4X)(R_5Q)P(=Z)][R_6]N-, [(R_4R_5N)(R_6X)P(=Z)][R_7]N-, [(R_4R_5N)(R_6R_7N)P(=Z)][R_8]N-, (R_4)(R_5X)P(=Z)-O-, (R_4)(R_5R_6N)P(=Z)-O-, (R_4)(R_5X)P(=Z)-S-, (R_4)(R_5R_6N)P(=Z)-S-, [(R_4)(R_5X)P(=Z)][R_6]N-, [(R_4)(R_5R_6N)P(=Z)][R_7]N-;

wherein the R_2 substituent can replace one or more hydrogens on any carbon atom of the naphtyl group, such as carbon atoms C1, C4, C5, C6, C7, and C8, provided that the carbon's valency is not exceeded;

wherein **X, Z and Q** are each, independently, O or S;

R_3 is R_4, R_4-C(=Z)-, R_4X-C(=Z)-, R_4R_5N-C(=Z)-, R_4O-S(=O)-, R_4O-$S(=O)_2$-, R_4R_5N-S(=O)-, R_4R_5N-$S(=O)_2$-, (R_4X)(R_5Q)P(=Z)-, (R_4R_5N)(R_6X)P(=Z)-, (R_4R_5N)(R_6R_7N)P(=Z)-;

wherein R_4, R_5, R_6, R_7 and R_8 are each, independently, hydrogen, C_{1-8}alkyl, C_{2-8}alkenyl, C_{2-8}alkynyl, C_{3-7}cycloalkyl, aryl, Het^1, Het^2;

each q present in formula (V) is, independently, 0, 1, 2, 3, or 4;

wherein any C_{1-8}alkyl, C_{2-8}alkenyl, C_{2-8}alkynyl or amino group, may be further mono, di-, or tri- substituted (if the valency allows it) with C_{1-4}alkoxy, C_{1-4}alkylcarbonyl, C_{1-4}alkoxycarbonyl, C_{1-4}alkylthio, C_{1-4}alkylsulfenyl, C_{1-4}alkylsulfinyl, C_{1-4}alkylsulfonyl, C_{1-4}alkylamino, halogen, nitro, azido, mercapto, sulfeno, sulfino, sulfo, cyano, amino, aryl, Het^1 or Het^2 substituents;

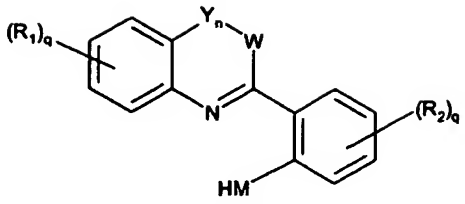
wherein the **BLOCKING GROUP** is a mono- or polysaccharide derivate, phosphate derivate, sulfate derivate, carboxylic acid derivate, or oligopeptide derivate;

with the proviso that at least one of R_1, R_2 and R_3 is C_{4-8}alkyl, C_{4-8}alkenyl, or C_{4-8}alkynyl.

7. Use of a substrate according to any one of claims 1 to 6, for permeation through the membrane of a biological cell.

8. Method for preparing a substrate according to any one of claims 1 to 6 comprising the steps of:
   - synthesizing a blocking group, whereby said blocking group may be optionally protected;
   - synthesizing a substituted fluorophore;
   - coupling the optionally protected blocking group to said substituted fluorophore;
   - optionally deprotecting said blocking group; and
   - purifying the resulting substituted substrate.

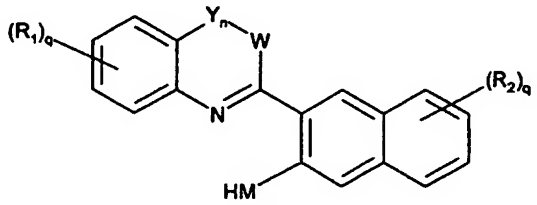
9. A fluorescent precipitate obtainable by cleavage of the **BLOCKING GROUP** moiety from the substrate of formula (I), (II), and (III) of any one of the claims 1 to 5, having the formula (IV)



(IV)

wherein **Y, n, W, M, R_1, R_2 and q** are as defined as in any one of claims 1 to 5.

10. A fluorescent precipitate obtainable by cleavage of the **BLOCKING GROUP** moiety from the substrate of formula (V) of claim 6, having the formula (VI):



(VI)

wherein **Y, n, W, M, R_1, R_2 and q** are as defined as in claim 6.

11. Method for detecting the activity of an enzyme comprising the steps of:
- contacting a sample containing said enzyme with a substrate according to any one of claims 1 to 6;
- applying conditions suitable to allow formation of a fluorescent precipitate according to any one of claims 9 to 10; and
- quantitatively or qualitatively analyzing said fluorescent precipitate.

12. Method according to claim 11 wherein analyzing said fluorescent precipitate comprises the steps of:
- exposing the fluorescent precipitate to a light source capable of producing light at a wavelength of absorption of the fluorescent precipitate; and
- detecting the resultant fluorescence of the precipitate.